permitted the nitrile symmetric stretch vibration of these UAAs to be unambiguously assigned utilizing the magnitude and direction of the isotopic shift of this vibration. The sensitivity of the nitrile symmetric stretching frequency of each isotopic variant to local environment was measured by individually incorporating the probes into two distinct local environments of sfGFP. The UAAs were also utilized in concert to probe multiple local environments in sfGFP simultaneously to increase the utility of 4-cyano-L-phenylalanine.

3520-Pos Board B675
2-Deuterated Histidine is a Raman Reporter of Histidine's Protonation State, Hydrogen Bonding, and Metal Coordination
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The C-2 proton on the imidazole side chain of the amino acid Histidine (His) can be isotopically exchanged for deuterium under mild reaction conditions, thus creating a unique C-D stretching band that can serve as a site-specific reporter of the environment and role of specific His residues in proteins. This band is a very weak infrared band but is clearly visible in nonresonant Raman spectra. His residues can participate in a number of different interactions unique to the His imidazole ring. Model compound work has shown that the peak frequency and spectral properties of this novel C-D vibrational band is dependent on imidazole protonation state, hydrogen bond donor and acceptor strength, and metal coordination, while salt bridge dependence in the model protein T4 lysozyme is currently being studied. Based on protein work that confirms the model compound protonation dependence, larger and more complex protein systems with multiple, distinct His residues, like superoxide dismutase, are currently targeted to report the environment and interactions of multiple specific His residues within the same protein.

3521-Pos Board B676
The Water Combination Band Reports Solute-Induced Changes in the Dynamic Structure of Bulk Water
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The bend+libration combination band in the infrared spectrum of H2O has important consequences for IR spectra of aqueous samples and for the dynamics of bulk water. The combination band has been shown to respond to solutes and even solvated proteins, complicating baseline subtractions in the region from 1900-2400 cm-1 that is the frequent target of biomolecule-based vibrational probe groups. Due to the less prominent and broad shape of this band, it has been largely overlooked in IR studies of water behavior and dynamics. However, librational motion of water molecules has been shown to be the primary force behind hydrogen bond exchange dynamics, while the bending motion can participate as well. Given the ps-scale of hydrogen bond-forming in liquid water, this band reports directly on these dynamic events through both vibrations, providing a surprisingly clear picture of molecule-scale dynamics in the structure of liquid water. The relationship between the effects of temperature and a range of different solutes on this band is presented here in the first comprehensive study of this band's solute dependence. The effects of denaturants, osmolytes, and solvated salts representing the full extent of the Hofmeister series, among other solutes, will be discussed.

3522-Pos Board B677
Heat Transfer Pathways in Proteins
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Proteins propagate excess thermal energy surprisingly fast and efficient. The theoretical description of thermal transport dynamics in proteins is challenging as it involves both fast quantum vibrations as well as more classical slow conformational changes. Existing protein Molecular Dynamics methods describe these conformational changes well but perform poorly for fast quantum degrees of freedom. Dye labeled Azido-PEG oligomers are experimentally well studied model systems for excess energy transport. Relaxation-assisted two-dimensional infrared (RA 2DIR) spectroscopy has been applied 1) 2) to measure the time-dependent correlation of frequencies belonging to the dye and the Azido group.
Thermal energy is generated at the dye and propagates through the flexible oligomer chain (intramolecular) as well as into the solvent (intermolecular). The oligomer chain undergoes conformational changes on the ps time scales of the transfer process. These slow changes were sampled using Molecular Dynamics trajectories. The resulting structural ensemble was used to map the mode coupling pathways for the fast quantum degrees of freedom. All pairwise mode-mode coupling potentials along pairs of dynamic normal mode vectors were calculated. A molecular heat map was generated using the spatial delocalization of the modes and the inter mode coupling strengths. Molecular heat maps have the potential to extend the theoretical understanding of intramolecular heat transduction pathways in proteins.
1) Lin, Z., Rubtsov, I.V., PNAS, 2012, 105, 5, 1413-1418
2) Lin, Z., Rubtsov, I.V., et al., Phys. Chem. Chem. Phys., 2012, 14, 10445-10454

Bioengineering

3523-Pos Board B678
Rational Design of a Zinc Phthalocyanine Binding Protein
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Phthalocyanines have long been used as primary donor molecules in synthetic light-powered devices due to their superior properties when compared to natural light activated molecules such as chlorophylls. their use in biological contexts, however, has been severely restricted due to their high degree of self-association, and its attendant photoquenching, in aqueous environments. To this end we report the rational redesign of a de novo four helix bundle di-heme binding protein into a heme and Zinc phthalocyanine dyad. The stepwise design pathway included the sulfonation of the phthalocyanine cofactor to destabilize stacking interactions, the creation of a single chain helical bundle protein in order to enable the creation of asymmetric binding sites and the removal of a disulfide bond to increase protein flexibility. The final design tightly binds one heme and one zinc phthalocyanine as a monomer in ''splendid isolation''. Singular binding of Zinc Phthalocyanine was verified by absorption, fluorescence and magnetic circular dichroism spectroscopies.

3524-Pos Board B679
Photo-Regulation of Small G Protein Ras Mutants using Photochromic Molecule
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Ras is one of small G-proteins known as a molecular switch mediating cellular signalling. Switching on state of Ras is induced by exchange of bound GDP for GTP and off state is by hydrolysis of GTP to GDP. Interestingly, the core nucleotide-binding motif of Ras is considerably conserved with the ATP driven motor proteins, myosin and kinesin. Therefore, it is believed that these biomolecular machines share common molecular mechanism utilizing nucleotide hydrolysis cycle. Previously, we have incorporated photochromic molecules, 4-phenylazophenyl maleimide (PAM), into the functional site of kinesin as a photo-switching device and succeeded to regulate kinesin ATPase activities reversibly upon visible light (VIS) and ultra-violet (UV) light irradiation. Therefore, it is expected that Ras can be also regulated by the similar method using photochromic molecules.
In this study, we performed basic study to control the function of Ras reversibly using photochromic molecules upon VIS and UV light irradiations. First, in order to monitor the exchange of bound GDP for GTP, we synthesized a new fluorescent GTP analogue, NBD-GTP, and GDP analogue, NBD-GDP, which change their fluorescent intensity along the formation of Ras-GTP, Ras-GDP-Pi and Ras-GDP states. And the GTPase activity of Ras was monitored by the quantitative analysis of GTP and GDP in the active site of Ras using reverse phase column chromatography on HPLC. We have designed three kinds of Ras mutants K5C, I36C, and Y64C. The mutants were prepared using *E.coli.*expression system and modified with PAM. It was suggested that the GTPase activities of Ras mutants modified with PAM were reversibly alternated upon ultra-violet and visible light irradiation.

3525-Pos Board B680
A Lattice Model of Cross-Linked Polymeric Materials: The Role of Frustration
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The diversity of properties of cross-linked polymeric materials cannot be explained by the constituent polymer chains alone but is attributed in part to their cross-linking. We propose a three-dimensional lattice model of bulk, polymeric materials that consist of stiff, cross-linked chains. Each chain has a randomly distributed, fixed number of binding sites that are capable of forming cross-links, which we call "active binding sites" (ABSs). The number of ABSs per unit length of chain, their interaction strength, and their distribution are the key parameters by which variability is achieved in representative materials. Inevitably, systems with a random distribution of ABSs retain unsatisfied bonds. We refer to these as "frustrated" systems. Numerically, an interfacial crossover is observed from an ordered state at low temperature to a disordered state at high temperature; the crossover scale depends on ABS density. Randomness

may therefore provide organisms with a method of varying materials properties while avoiding critical oscillations.

3526-Pos Board B681
An Active Gel based on DNA and DNA-Associated Motor Proteins
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Biological systems continuously acquire and use energy sources to perform various functions. This energy is, in part, transduced to generate the forces that control the mechanical behavior of the cell (e.g. cell shape and motion). In this case, the system is in the non-equilibrium state and the material may be called ''Active Soft Matter''.
To investigate the mechanical properties of soft, active systems, we have synthesized an active gel with a well-known semi-flexible biopolymer, DNA, and DNA-associated motor proteins. We study the mechanics of this system using two kinds of microrheological techniques. First, we use a passive measurement in which the intrinsic fluctuation of embedded particles gives information on gel mechanics. Second, we use an active measurement utilizing the forced oscillating motion of embedded particles by an external magnetic field. We discuss these results in comparison to cytoskeletal systems, and seek to establish universal principles of motor-driven active gels.

3527-Pos Board B682
Designing Microdevices Operated through Self-Organizations of Microtubules and Kinesin Motors
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Fish melanocytes change their colors through aggregation and dispersion of melanophores. The aggregation and dispersion of melanophores make the appearances of fish melanocytes bright and dark, respectively. The movements of malanophores are driven by biological molecular motors, motor proteins. Inspired by this mechanism, we have envisioned an optical microdevice powered by motor proteins. That is, in arrays of microscale chambers, melanophore-imitated particles, whose surfaces are covered with kinesin motors, are aggregated and dispersed through formations and disassemblies of microtubule asters, respectively. In order to realize such optical device, exploring possible designs of the device is required to test the feasibility of the device. However, laborious experimental procedures hamper such explorations. An alternative way of the exploring would be use of computer simulations. Previously, we have shown the power of computer simulations in designing Lab-on-a-Chip devices powered by motor proteins. Here, we performed systematic explorations of designs of the envisioned device. To this end, we modeled a microtubule as the Kramers chain of a linear polymer, and performed Brownian dynamics simulations. The simulations showed aster formations of microtubules in chambers of various shapes, such as thin triangle, square and hexagonal prisms. We will discuss effects on the aster formations of the size of microscopic chambers, microtubule properties, and motor protein properties. Through the computer simulation, we will show design guidelines for the optical device.

3528-Pos Board B683
Concurrent Transcript and Protein Quantification in a Massive Single Cell Array Enables Population-Wide Observation of Oncogene Escape
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The cancer stem cell (CSC) model focuses on the key role these cells can play in drug resistance, since CSCs are believed to reconstitute the cancer after intense chemotherapy treatment. In order to effectively identify and profile CSCs within a heterogeneous tumor population, we are investigating quantitative correlation of messenger RNAs and their translated proteins as a distinctive parameter of the CSC population. However, previous research on mRNA expression and protein abundance has shown the correlations between the two are weak or only ''stochastically meaningful'' due to the significant level of experimental error originating from ensemble observations. Here, we demonstrate a robust microwell-based method to minimize these errors by monitoring both mRNA expression and the corresponding protein abundance from individual cancer cells. Simultaneous observation of membrane protein expression by immunostaining and detection of mRNA transcripts directly from individual cells using a one-step, reverse transcription polymerase chain reaction have been integrated in a massive single-cell array platform. The proposed experimental scheme was initially tested and validated in three established lung cancer cell lines by correlating mRNA transcript and protein expression levels of individual cells and quantitation of heterogeneity. Responses at the individual cell level to known transcriptional and translational inhibitors, as well as to EGFR-specific inhibitors were evaluated, providing quantitative measures of the heterogeneous response of non-small cell lung cancer cells to the inhibitors. Results showed that drug-treated cell lines displayed oncogene escape due to expunction of drug-sensitive subpopulations in the cell lines. Furthermore, correlation of c-MET mRNA and protein levels revealed unique response patterns in different EGFR-mutated cell lines. Thus, these results demonstrate the potential for molecular profiling at the single cell level to prospectively identify the CSCs subpopulation for effective combinatorial treatments.

3529-Pos Board B684
Enhancing Automatic Mass Detection in Ultrasound Breast Images using Computer Assisted Detection
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This work is concentrated on extraction of mass in Ultrasound breast images to help radiologists interpreting such images efficiently using Computer-Assisted Detection. A set of six popular ultrasound machines were selected and images were acquired sweeping: modes of operation, transducer, frequency and contrast. To make a complete set of ultrasound images in B-Mode a multi purpose multi tissue Ultrasound Phantom was used. Gamma corrections, contrast stretching and filtering accompanied by morphological Image Processing were among the steps that were applied to find the final image. Two experienced radiologists were marked output. Statistical analysis showed a sensitivity of 100% and accuracy of 99% for solid mass and 99% and 98% for cystic mass respectively. It also showed that the same procedure can be use for cystic and solid breast masses with small changes.

3530-Pos Board B685
Modulation of the Invasive Phenotype of Engineered Breast Tumors by the Physical and Cellular Microenvironment
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Tumor development alters the normal cellular processes that maintain tissue integrity and homeostasis, and introduces changes to the tissue surrounding the tumor as well as the tumor cells themselves. Tumor development and invasion are regulated by the physical and chemical properties of the interstitial microenvironment. Here, we examined the effects of both interstitial fluid pressure and vascular endothelial cells on the invasive phenotype of engineered three-dimensional (3D) aggregates of MDA-MB-231 human breast cancer cells. The directionality of the interstitial pressure profile and the presence of endothelial cells altered the frequency at which cells invaded from the surface of the aggregate. Moreover, introducing pressure at one end of an aggregate suppressed invasion at the opposite end. We found previously that elevated interstitial pressure inhibits invasion by altering the chemical composition of the interstitial fluid near the surface of the aggregate. Our data reveal a link between hydrostatic pressure, the vascular endothelium, interstitial convection, and invasion.

3531-Pos Board B686
Fibrillar Collagen is Equivalent to Stiff Matrix in Driving Marrow Stromal Cell Differentiation into a Matrix-Deficient, Myofibroblastic-Like Phenotype
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Scars tend to be stiffer than normal tissue, which has prompted the use of stiff matrices as models of scars, but scars are also rich in fibrillar collagen-I. Here, we introduce a soft matrix embedded with distinctly fibrillar collagen type-I, and show that this is sufficient to drive bone marrow stromal cells (MSCs) into a contractile, myofibroblastic-like phenotype – 'myo-MSCs'. These cells have been reported to minimize scarring in a unique wound healing response, exemplified by their application to myocardial infarcts. Transcriptome analysis in response to matrix rigidity points to an upregulation of genes that participate in the cellular contractile machinery, notably α-smooth muscle actin (SMA), but a decreased expression of matrix protein genes for collagen types I and VI, and tenascin-C; TGFβ1 and TGFβRII, implicated in progressive fibrosis,